

Figure 1 (A-F)

Construct Forms Comprising at Least one Single-Stranded Region

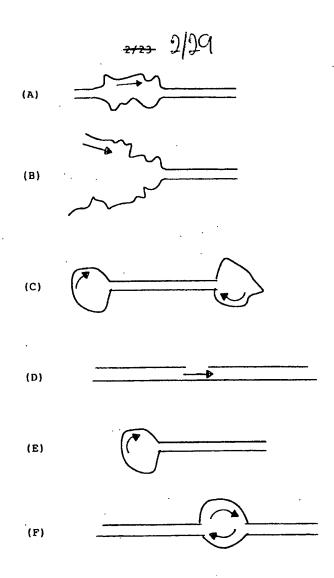
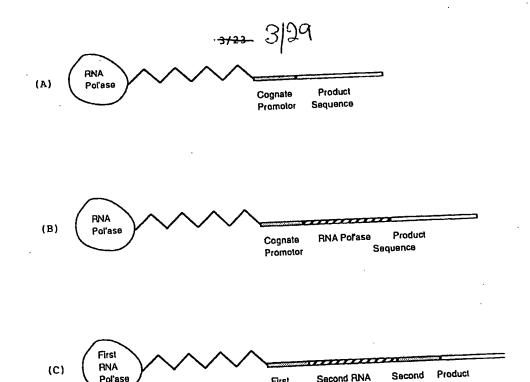


Figure 2 (A-F)

Functional Forms of the Construct



Pol'ase

Figure 3 (A-C)

First

Promotor

Second RNA

Pol'ase

Promotor Sequence

Three Constructs with an RNA Polymerase Covalently Attached to a Transcribing Cassette

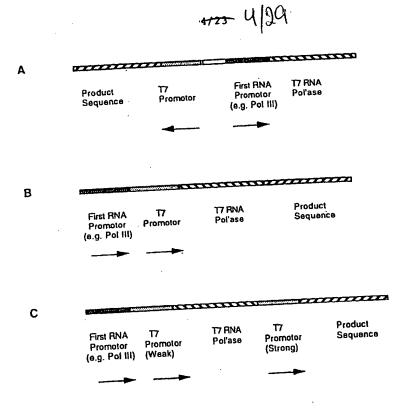


Figure 4 (A-C)

Three Constructs with Promoters for Endogenous RNA Polymerase

M13mp18. Seq Length: 7250

M13mp18. Seq Length: 7250
1. AATECTACTA CTATTAGTAG AATTGATGOC ACCTTTTCAG CTOGOGOCOC
51. AAATGAAAAT ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA
101 ATGGTCAAAC TAAATCTACT OGTTOGCAGA ATTGGGAATC AACTGTTACA
151. TOGAATGAAA CTTOCAGACA COGTACTITA GTTGCATATT TAAAACATGT
201 TGAGCTACAG CACCAGATTC AGCAATTAAG CTCTAAGCCA TOOGCAAAAA
251 TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG
301. TIGGAGITIG CITICOGGICT GGITIOGCITT GAAGCIOGAA TTAAAACGOG
351. ATATTIGAAG TCTTTCCCCTTAA TCTTTTTGAT GCAATCOGCT
TOTAL CTATAATAGT CAGGGTAAAG ACCTGATTIT TGATTTATGG
THE THE THE TRANSPORT OF THE PROPERTY OF THE P
THE TOTAL CATHOGRAG TATIGGACGC TATOCAGTCT ASACATTITA
THE TARGET CHEIRSCAAA ACTICITITG CAAAAGOCTC TOGCTATTIT
STATE CHARGE AMAGEMENT TATGATAGTS TIGCTICITAC
AATTOCTITI GEOGITATET ATCIGCATTA GITGAATETE
ATCANTCHIT CTACCIGIAA TAATGTIGTT
701. GIATICOMA AIGUSTA COTACATETE TOTIOCCAAC GIOCIGACIG
751. COGITAGIIC GITTATIAN CONTRATA ACCTAATICA CAATGATTAA
801. GTATAAIGAL COAGITOTA TOTAATT TACTACTOST TCTOGTGTTC
851. AGTTGAAATT AAACCAICIC AAGCCATT
901. TOGTCAGGGC AAGCTIATI CACIGAAGGTCA
951. TOOGTAATGA ATATOOGGT CHIGHOUSE TOTTTO TOTTTO TOTTTO TOTTTO AAAG
1001 GOCAGOCTAT GOGOCTEGIC IGIALACOGI IGIALACOGI
1051 TIGGTCAGTT COGTICCULT AIGANIGACO CITOLOGICA
1101 AAGTAACATG GAGCAGGIUG USGAITIUGA CACATTITUS
1151 TACAAATCTC CGTTGTACCTT TGTTTCQCQC TTGGTATAAT CGCTQCCCGT
1201 CAAAGATGAG TGTTTTAGTG TATTCTTTCG CCTCTTTCGT TTTAGGTTGG

Figure 5

6/22 6/29

CTICSCATTAC GTATTITIACC OGTITAATEG AAACTTOCTC
1251 TGCCTTCGTA GIGGLATIAN CHARCOSTIG CTACCCTCGT
1301 ATGAAAAAGT CTTTAGTCCI CAMACCICI CAMACCICIC CAMACCIC
1351 TOOGATECTG TCTTTOGCTG CIGAGGGIGA COALCOCK
1401 TTAACTOOCT GCAAGOCTCA GUSACCAA
1451 ATGGTTGTTG TCATTGTCGG CGAACIAGO
1501 ATTCACCTCG AAAGCAAGCI GAIAAAACCA IAAAAACCA
1551 GGAGOCTTTI TTTTTGGAGA TTTTCAACGT GAAAAAATTA TTATTCGCAA
1601 TICCITIAGI TIGTICCITIC TATTICTCACT COGCIGAAAC IGIIGAAAGI
ANYTHATAC AGAAAATICA TITACTAACG TCTGGAAAGA
1651 TGTTAGLAA AACCETTAGATOGTT ACGCTAACTA TGAGGGTTGT CTGTGGAATG
1701 CONSTRUCTION ANACTORGIG TRADEGRACA
1/51 CHARLES THEORETICS TATOOCTICAA AATGAGGGTG GTGGCTCTGA
1801 ICCONTO
1851 GEGIGERALIA ICIGARENTE ATACTIATAT CAACOCICIC
1901 CIGAGIACAS TACTORCOM ACCORCTA ATOCTAATOC
1951 GACGGCACTT AILUGALCIGG FACTORIAGO TITICATGTTT CAGAATAATA
2001 TICTOTIGAG GAGICIOAGO CIOTAGA
2051 GGTTCCGAAA TAGGCAGGGG GLATTAACIG TTTTACACTC CTGTATCATC
2101 CAACCCACTG ACCCCGTTAA AACTTATTAC CALIFORNIA
2151 AMAGOCATG TATGACGCTT ACIGGAAGGG PARTIES
2201 CAAGGCACTG ACCOCGITAA AACTTATTAC COLOR
2151 AAAAGCCATG TGCCTCAACC TCCTGTCAAT GCTGGCGGGG GCTCTGGGGG
AND TOTATICIES CTITAATCAA GATOCATIOS TITISTISAATA ICAAGGOOM
TOCTICIONACE TOCTICAACE TOCTIGTCAAT GCTGGGGGGG GCTCTGGTGG
2251 TOCTIONES GEOGRAPHIC AGRICAGES CICIGAGEST GEOGRAPHICIG
2351 AGGIGGOGG CICIGAGGGA GGGGGITGGG GIGGIGGCIC TGGTTCGGT
2351 ASSESSED CTATGACCICA AATAAGGGGG CTATGACCGA
2401 GATTTO
2451 AAATGOOGAT GAAAACCCC Figure 5
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TOTAL STOTE TO TOTAL TOTAL STORE TO THE TOTAL STORE THE TOTAL S
2501 CTGTOCCTAC TGATTAOGGI GCIGCCTAG
2551 TOOGGOCTIG CTAATGGTAA TGGTGCTADT
2601 TTOOCAAATG GCTCAAGTOG GTGACGETGA TAATTCACCT TTAATGAATA
2651 ATTICOGTICA ATATTIACCT TOOCTOCCTC AATOOGTIGA ATGICOCCCT
27.0.1 TITIGTICTITA GOGCTEGTAA ACCATATGAA TITTICTATTIG ATTIGTGACAA
2751 AATAAACITA TIOOGIGGIG TCTTTGOGIT TCTTTTATAT GITGOCAOCT
2801 TTATGTATGT ATTTTCTACG TTTGCTAACA TACTGCGTAA TAAGGAGTCT
2851 TTATCATGCC AGTICTITIG GGTATICCGT TATTATTGCG TTTCCTCGGT
2901 TICCTICTIGG TAACTITIGTT COGCTATCTG CITACTITTIC TTAAAAAGGG
2951 CTICOGTANG ATAGCTATTG CTATTTCATT GITTCTTGCT CTTATTATTG
3001 GCCTTAACTIC AATTICTTGTG GCTTATCTCT CTGATATTAG CGCTCAATTA
SUUT COMMITTE TIGHT AGG TIGHTCAGTTA ATTICTCCCGT CTAATGCGCT
TATCHTATIC TCTCTGTAAA GGCTGCTATT TTCATTTTTG
AAAAATCGTT TCTTATTTGG ATTGGGATAA ATAATATGGC
CTANCTOCA AATTAGGCTC TOGAAAGACG CTOGITAGOG
- CETTS AAAT AGCAACTAAT
3251 TIGGIANGA COTOCOGCAA GTOGGGAGGT TOGCTAAAAC
3301 CHGATTAN CONTRACTOR TICTATATCT GATTIGCTIG
3351 GOCTOGOGII CITAGAATAC COCTOGOTI
3401 CTATTGGGGG CGGTAAIGAT ICCIACGATIG
3451 GTTCTCGATG AGIGLOGIAC FIGGITIAN AND AND AND AND AND AND AND AND AND A
3501 GGAAAGACAG COGATTATIG ATTGGTTTCT ACTGCTCGT AAATTAGGAT
3551 GGGATATTAT TTTTCTTGTT CAGGACTTAT CTATTGTTGA TAAACAGGCG
3601 COTTCTGCAT TACCTGAACA TGTTGTTTAT TGTCGTCGTC TCGACAGAAT
3651 TACTITIACCT THIGTOGGTA CTITATATTC TCTTATTACT GOCTOGAAAA
3701 TEOCTICIECC TAAATTACAT GTTEGOGTTG TTAAATATEG OGATTCTCAA
3701 TOSCITA CIETIRAGOS TIGOCITTAT ACTOGIAAGA ATTIGIATAA
3757 THE STATE ACTAMACAGE CITHTICTAG TAATTATGAT TOOGGIGHT
3801 CHAIAIGAI ADIMA

Figure 5

3851 ATTICTTATTT AACGOCTTAT TTATCACACG GTOGGTATTT CAAACCATTA ATTAACTAAA ATAATATTGA AAAAGTTTTC 3901 AATTTAGGTC AGAAGATGAA TIGTICTTOOGA TTIGGATTTOC ATCAGCATTT ACATATAGTT 3951 TOGOGTTCTT TCAGACCTAT **AGGTAGTCTC** ACCTANGEDES GAGGITTAAAA 4001 ATATAACCCA **ATCTAAGCTA** CAGOGTOTTA 4051 GATITIGATA AATTCACTAT TGACTCTTCT AGOGACGATT ATTAATTAAT CTAAGGGAAA 4101 TOGCTATGTT TTCAAGGATT TACTGTTTCC TTGATTTATG AGGITATICA CTCACATATA 4151 TACAGAAGCA AAATGTAATT AATTTTGTTT 4201 ATTAAAAAAG GTAATTCAAA TGAAATTGTT CTCAGGTAAT TGAAATGAAT 4251 TCTTGATGTT TGTTTCATCA TCTTCTTTTG AATCAGGCGA 4301 AATTOGOCTC TGOGGGATTT TGTAACTTGG TATTCAAAGC 4351 AATOCGITATT GITTICTOCCG ATGTAAAAGG TACTGTTACT GTATATTCAT TGTTTTACGT 4401 CTGAOGITAA AOCTGAAAAT CTAOGCAATT TCTTTATTTC 4451 GCTAATAATT TTGATAATGGT TGGTTCAATT CCTTCCATAA TTCAGAAGTA ATATTGATGA ATTGCCATCA TCTGATAATC 4501 TAATOCAAAC - AATCAGGATT TGTTCCGCAA ectecticis stestitett TGATAATTCC 4551 AGGAATATGA 4601 AATGATAATG TTACTCAAAC TTTTAAAATT AATAACGTTC CCCCAAACCA TCTAAATCCT 4651 TTTAATAOGA GTTGTOGAAT TGTTTGTAAA GTCTAATACT TAGTIGCTICCT GECTICTAATIC TATTAGTTGT 4701 CAAATGTATT ATCTATTGAC TTGATTTGCC CTTTCTACTG TOCTCAATTC TAGATAACCT 4751 AAAGATATTT CAGCAAGGTG ATTTGAGGTT ACCCITTICAT **ATATTGATTG** 4801 AACTGACCAG CACTGTTGCA CTCAGOGTGG **CONTRACT** TTTTCATTT 4851 ATGCTTTAGA CIECTEGIEG OCTCACCTCT GTTTTATCTT **ATACTGACCG** 4901 GEOGGIGITA GTTOGOGCAT AGGGCTATCA **GOGATGTTTT** ATTTTTAATG 4951 TTOGTTOGGT CTGTGCCACG TATTCTTACG **AAAATATTGT** TAGOCATTCA 5001 TAAAGACTAA TCCCTTTTAT **GGCCAGAATG** 5051 CTTTCAGGTC AGAAGGGTTC TATCTCTGTT TATTCTTACG CTGTGCCACG 5101 TAAAGACTAA TAGOCATTCA AAAATATTGT TOCTGTTGCA 5151 CGATTGAGCG TCAAAATGTA GGTATTTCCA TGAGOGTTTT

-Figure 5

9/23 9/29

5201 AT	COCTOCOCO .	GTAATATTGT.	TCTGGATATT	ACCAGCAAGG	COGATAGITT
5251 G	AGITOTOT	ACTICAGGICAA	GTGATGTTAT	TACTAATCAA	AGAAGTATTG
5,301 C	TACAAOGGT	TAATTTGCGT	GATGGACAGA	CTCTTTTACT	COGTICOCTIC
5351 A	CTGATTATA	AAAACACTTC	TCAAGATTCT	GGCGTACCGT	TOCTGTCTAA
5401 A	ATCCCTTTA	ATCCCCCCTCC	TGTTTAGCTC	COGCTCTGAT	TOCAAOGAGG
5451 A	AAGCACGTT	ATACGTGCTC	GTCAAAGCAA	CCATAGTACG	OCCOCTIGITAG:
5501 O	GCCCCATTA	ASOSSOSSOS	GIGIGGIGGI	TACCECCECACEC	GTGACCGCTA
5551 C	ACTTGCCAG	COCCTAGCG	COORCIOCIT	TOGOTTOTT	<u></u> σεποεπί
5601 C	TOGOCAOGT	TOGOOGCIT	TOXXXXTCAA	CCTCTAAATC	GEGEGETICCC
5651 T	TTAGGGTTC	CGATTTAGTG	CTTTACCGCA	CCTCCACCCC	AAAAAACTTG
5701 A	ATTEGGTGA	TEGTTCACGT	AGTGGGCCAT	COCCTGATA	GACGGTTTTT
5751 C	CCCTTTGA	CCTTCCACTC	CACGITICITIT	AATAGTGGAC	TCTTGTTCCA
5801 A	VACTGGAACA	ACACTCAACC	CTATCTOGGG	CTATTCTTTT	GATTTATAAG
5851 (2CATTTTCCC	GATTTOGGAA	CCACCATCAA	ACAGGATTTT	CECCTECTEG
5901 (23CAAACCAG	CCTTCCACCCCC	TTGCTGCAAC	TCTCTCAGGG	CCAGGCCGTG
5951	AAGGGCAATC	AGCTGTTGCC	OCICIOSCIG	GTGAAAAGAA	AAAOCAOOCT
6001	CCCCCCCC	ACGCAAACCG	CTCTCCCCCG	COCCTTCCCC	GATTCATTAA
6051	TECAGCTEGEC	ACCACAGGIT	TOOGACTEG	AAAGOGGGCA	GTGAGCGCAA
6101	COCAATTAAT	GTGAGTTAGC	TCACTCATTA	CCCACCCCAG	GCTTTACACT
6151	TTATGCTTCC	GECTOGTATG	TIGIGIGGAA	TIGIGAGOGG	ATAACAATTT
6201	CACACAGGAA	ACAGCTATGA	CCATGATTAC	GAATTOGAGO	TOGGTACCOG
6251	GOGATOCTCT	AGAGTOGACO	TOCAGOCATO	CAAGCTTGGC	ACTEGEOCETC
	GTTTTACAAC		GGAAAACCCT	GEOGITACCC	AACTTAATOG
6351	CCTTGCAGCA	CAATCCCCTT	TOGOCAGCTG	GOGTAATAGO	GAAGAGGCCC
6401	GCACCGATCC		CÁGILIGOGCA	COCTGAATOC	CGAATGGCCC
6451	THEOCIEG		AGAAGOGGTO	COGGAMAGIC	COCTOCAGIG
6501	OGATCTTOC*		A COGNICON	r cooctcaaa	TEGECAGATEC
9901	٠٠٠.٠٠				•

Figure 5

10/23 10/29

6551	ACGGTTACGA	TGCGCCCATC	TACACCAACG	TAACCTATCC	CATTACGGTC
6601	AATCCGCCGT	TIGITCOCAC	CCACAATOOG	ACCECTIGIT	ACTOGCTCAC
6651	ATTTAATGTT	GATGAAAGCT	GGCTACAGGA	AGGCCAGACG	CGAATTATTT
67 [.] 01	TTGATGGCGT	TOCTATTGGT	TAAAAAATGA	GCTGATTTAA	CAAAAATTTA
6751	ACCCCAATTT	TAACAAAATA	TTAACGTTTA	CAATTTAAAT	ATTTGCTTAT
6801	ACAATCTTCC	TGTTTTT@GG	GCTTTICTGA	TTATCAACOG	GGGTACATAT
6851	GATTGACATG	CTAGTTTTAC	GATTACCGTT	CATCGATTCT	спаптест
6901	CCAGACTCTC	AGGICAATGAC	CTGATAGOCT	TTGTAGATCT	CTCAAAAATA
6951	GCTACCCTCT	COCCCATGAA	TTTATCAGCT	AGAACGGTTG	AATATCATAT
7001	TGATGGTGAT	TTGACTGTCT	OCCEPTION TO	TCACCCTTTT	GAATCTTTAC
7051	CTACACATTA	CTCAGGCATT	GCATTTAAAA	TATATGAGGG	TTCTAAAAAT
7101	TTTTATCCTT	GOGTTGAAAT	AAAGGCTTCT	CCCCCAAAAG	TATTACAGGG
715	TCATAATGTT	TTTGGTACAA	COGATTTAGC	TITATGCTCT	GAGGCTTTAT

Figure 5

11/23 11/29

COMPLEMENTARY TO M₁₃

POSITION 6 4 5	5 ' 3' AGCAACACTATCATA	POSITION 631	M ₁₃ /1
615	ACGACGATAAAAACC	601	M ₁₃ /2
585	TTTTGCAAAAGAAGT	571	M ₁₃ /3
555	AATAGTAAAATGTTT	541	M ₁₃ /4
525	CAATACTGOGGAATG	511	M ₁₃ /5
495	TGAATCCCCTCAAA	481	M ₁₃ /6
465	AGAAAACGAGAATGA	451	M ₁₃ /7
435	CAGGTCTTTACCCTG	421	M ₁₃ /8
405	AGGAAAGOGGATTGC	391	M ₁₃ /9
375	AGGAAGOOOGAAAGA	361	M ₁₃ /10

COMPLEMENTARY TO SS PHAGE DNA

POSITION	•	POSITION	
351	5' • • 3' ATATTTGAAGTCTTT	366	M ₁₃ /11
371	TCTTTTTGATGCAAT	386	M ₁₃ /12
391	CTATAATACTCAGGG	406	M ₁₃ /13
411	TGATTTATGGTCATT	426	· M ₁₃ /14
431	GTTTAAAGCATTTGA	446	M ₁₃ /15
451	TATTTATGACGATTC	466	M ₁₃ /16
471	TATOCAGTCTAAACA	486	M ₁₃ /17
491	CTCTGGCAAAACTTC	506	M ₁₃ /18
511	TOGOTATTITIGGTTT	526	M ₁₃ /19
-531	AAAOGAGGGTTATGA	546	M 13/20

Figure 6

Primers for Nucleic Acid Production Derived from M13mp18 Sequence

12/23/2/29

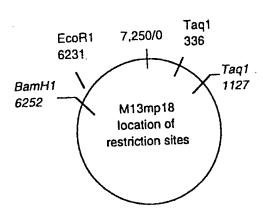


Figure 7

Appropriate M13mp18 Restriction Sites

13/23- 13/29



Lane 1: from calf thymus + Taq digested mp18 amplification reaction

Lane 2: from Taq digested mp18 amplification reaction

Lane 3: from calf thymus amplification reaction

Lane 4: ØX174 Hinf1 size marker

Figure 8

14/23 14/29



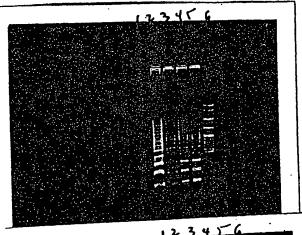
Lane 1: no template

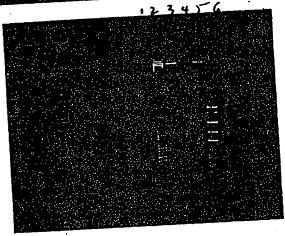
Lane 2: mp18 template, phosphate buffer

Lane 3: Mspl/pBR322 size marker Lane 4: mp18 template, MOPS buffer

Figure 9

15/23 15/29





Top= (+) Template
Bottom= (-) Template

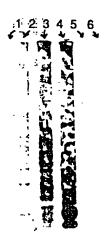
Lane 1: phosphate buffer

Lane 2: MES Lane 3: MOPS Lane 4: DMAB Lane 5: DMG

Lane 6: pBR322/Mspl size marker

Figure 10

16/23 16/29



Lane 1: DMAB buffer, no template

Lane 2: DMAB buffer, mp18 template

Lane 3: DMG buffer, no template

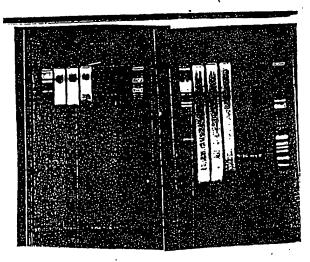
Lane 4: DMG buffer, mp18 template

Lane 5: No reaction

Lane 6: 200 ng Taq I digested mp18 size marker/positive control

Figure 11

17/23 17/29



First Time Interval Second Time Interval

Agarose Gel Analysis

Lane 1: lambda Hind III marker

Lane 2: Amp/Untreated

Lane 3: Amp/Kinased

Lane 4: Amp/Kinased/Ligated

Lane 5: PCR/Untreated

Lane 6: PCR/Kinased

Lane 7: PCR/Kinased/Ligated

Lane 8: øX174/Hinf1 marker

Figure 12

18/23 18/29

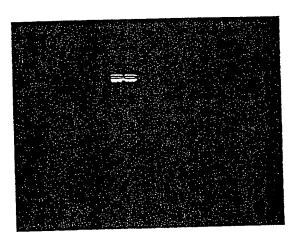
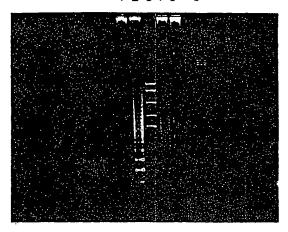


Figure 13

19/23 19/29

1 2 3 4 5 6



Lane 1: Primers alone

Lane 2: Primers + taq digested M13 DNA

Lane 3: Molecular weight markers

Lane 4: Primers + RNA

Lane 5: Primers alone

Lane 6: M13 digested DNA

Buffer was dimethyl amino glycine, pH 8.6

Figure 14

20/23 20/29



Lane 1: Primers alone

Lane 2: Primers + taq digested M13 DNA

Lane 3: Molecular weight markers

Lane 4: Primers + RNA Lane 5: Primers alone

Lane 6: M13 digested DNA

Buffer was dimethyl amino glycine, pH 8.6

Figure 15

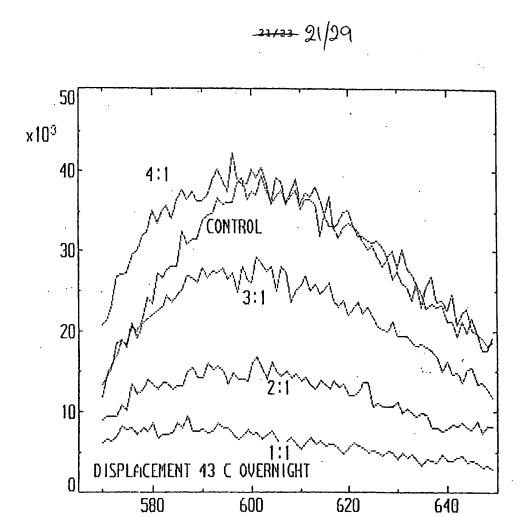


Figure 16

Wavelength (nm)

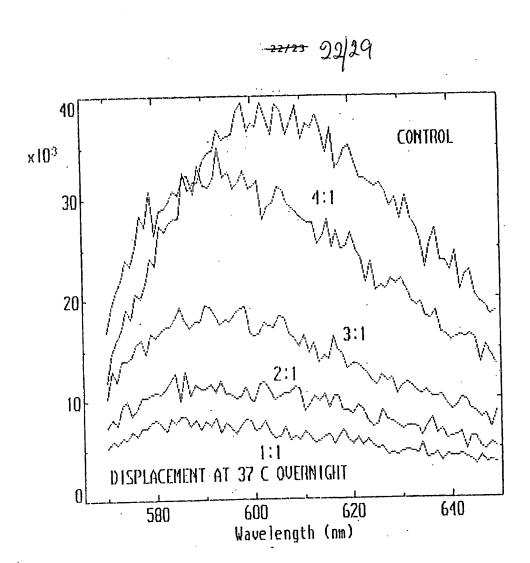


Figure 17

-23/23 23/29

pIBI 31-BH5-2

fmet AUG of Lac z {T7 Promotor region---LAC PROMOTOR.ATG ACC ATG ATT ACG CCA GAT ATC AAA TTA ATA CGA CTC ACT ATA

oligo 50-mer

3'- tac t'aa t'gc ggt' ct'a t'ag t'Vt aat' tat' gct' gag t'ga t'at' c-5' 10 base insert

T7 RNA Start («« T3 Promotor Region)
IGGG CTC ICCT TTA GTG ACG GTT AAT
----»») «- T3 Start Signal

pIBI 31 BSII/HCV

Imet AUG of Lac z [T3 Promotor region --»] T3 RNA Start LAC PROMOTOR ..ATG ACC ATG ATT ACG CCA AGC TCG AAA TTA ACC CTC ACT AAA /QQG oligo 50-mer 3'- tac t'aa t'ac t'aa t'gc ggt' t'V--10 base insert-----------------------

{«- T7 Promotor Region }

MULTIPLE CLONING SITE + 390 BASE INSERT CTA /TAG TGA GTC CGT ATT AAT....

«- T7 Start Signal

5'-ct'a t'ag t'ga gt'c gt'a tt'a at'...........

24/29

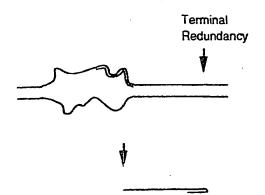
25/29

Replication Bubble with Nucleotide Analogs



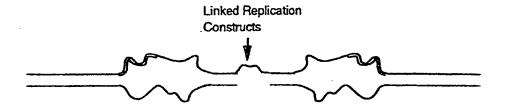
Primer-Dependent DNA Production Using Nucleic Acid Construct

26/29



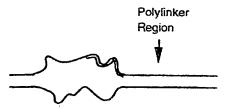
Hairpin Product

27/29



Linked Complementary Production Constructs

27/29



Cloning Site in Production Constructs

29/29

ARRANGEMENT OF OLIGONUCLEOTIDE PRIMERS IN AMPLIFICATION REACTION

1	2	3	4	5	6	7	8	9	10
20	19	18	17	16	15	14	13	12	11

Figure 24